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Nuclear Paracrystals in the Fern *Polystichum setiferum* Gametophyte

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ABSTRACT.—Single nuclear paracrystals were observed in gametophyte cells of the fern *Polystichum setiferum*. They consist of proteins poor in basic aminoacids and have sharp-profiled, polyhedral shapes, with inner diameters up to 4.5 μm. The substructure is a fine, very close-meshed reticulum resolvable at magnifications of 40,000× or more. The hypothesis that these paracrystals are storage bodies of unused nuclear proteins is discussed.

Proteinaceous nuclear paracrystalline formations have been described in animals and plants. For example, in plants they have been found in: algal cells (Pickett-Heaps, 1967; Barton, 1967); meristematic cells of the root tip of different species of Pteridophyta (Fabbri and Menicanti, 1970); leaf cells of ferns (Weintraub et al., 1971; Fabbri and Menicanti, 1970) and an angiosperm (Perrin, 1969, 1970); photosynthesising stem cells of a fern (Menicanti, 1972); sieve elements of ferns in the Polypodiaceae (Hébant, 1969; Evert and Eichhorn, 1974) and angiosperms in the Boraginaceae (Esau and Thorsch, 1982; Thorsch and Esau, 1983); and embryo, style, and stigma cells of angiosperms (Villiers, 1968; Ciampolini *et al.*, 1983). Nuclear crystalloids have never been reported in fern gametophytes. This paper reports the electron microscopic evidence of these functionally enigmatic formations in nuclei of gametophyte cells of *Polystichum setiferum*, a species that is diffusely widespread in Europe (Tutin et al., 1993). In Italy, it is present in Alps, Pre-Alps and tyrrhenian regions (Ferrarini et al., 1986).

MATERIALS AND METHODS

Polystichum setiferum (Forssk.) T. Moore ex Woynar spores were sterilized in 1% sodium hypochlorite for few minutes. Spores were sown on Knop's medium modified according to Vaudois and Tourte (1979) in sterilized Petri plates and were exposed to "daylight" fluorescent tubes at 23°C on a 12/12 h light/dark cycle. Fragments of mature gametophytes were fixed in Karnowsky solution for 2 h at 4°C and post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2) for 2 h at 4°C in the dark. They were then dehydrated in ethanol and embedded in Epon 812-Araldite A/M mixture (Mollenhauer, 1964). Thin sections were stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). Sections 1–2 μm thick of samples processed for electron microscopy were stained for proteins at 45°C with 0.5% Ponceau 2R in 2% periodic acid solution at pH 1.5 (Gori, 1978).

RESULTS AND DISCUSSION

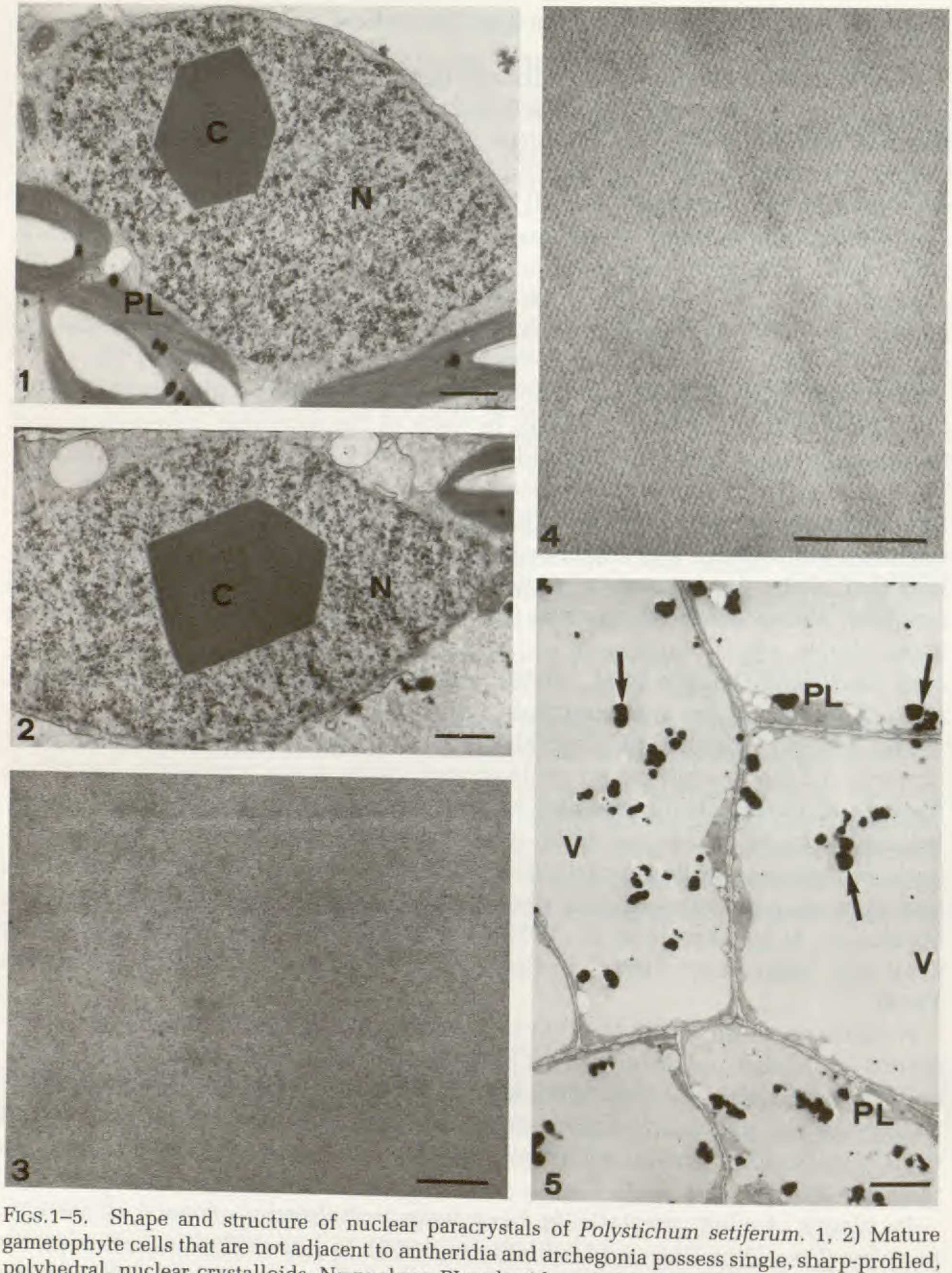
Under controlled culture conditions, four months after spores of *Polystichum setiferum* are sown, a cordate bisexual gametophyte with large tightly packed cells develops. The interphase nuclei have diffuse chromatin and are ellipsoidal, with mean longitudinal and transverse diameters of 7 and 10 μm respectively. Most of them—i.e., all except those of cells adjacent to antheridia and archegonia—contain single paracrystals (Figs. 1, 2). These paracrystals have sharp, polygonal outlines indicating regular polyhedral shapes. The diameters are up to 4.5 μm. The inner structure is a very close-meshed reticulum clearly resolvable at magnifications of 40,000× or more (Figs. 3, 4). Unlike the nucleus and nucleolus, the crystalloids are not stained by Ponceau 2R.

In *P. setiferum,* nuclear crystalloids have been reported previously in leaf cells (Fabbri and Menicanti, 1970). In light of the available information and the present study, both the haploid and diploid phases of the species clearly possess nuclear paracrystals. In leaf cells, the nuclear paracrystals are very rare, whereas in gametophyte cells they are abundant. Both have polygonal outlines, but those of leaf cells have a maximum diameter of 1.9 μm, less than half that of the paracrystals of gametophyte cells. Unlike those in *Pinguicola caudata, Polypodium punctatum* (Sauer, 1962, cited by Frey-Wyssling and Muhlethaler, 1965), *Asplenium nidus, Polypodium aureum,* and *Campyloneurum phyllitidis* (Wergin et al., 1970), nuclear paracrystals of *Polystichum setiferum* gametophytes are unrelated to nucleoli in size.

The sharply angular, polyhedral shape and close-meshed, reticulate substructure of the present paracrystals are two of the many previously described features of these cellular entities. Nuclear inclusions vary in shape from polygonal to more-or-less regularly spherical bodies, and the substructure has been reported as latticed, crystalline, fibrous, tubular, granular, amorphous, and even as consisting of only one or two distinct components (Fabbri and Menicanti, 1970; Wergin et al., 1970; Weintraub et al., 1971; Menicanti, 1972; Esau and Magyarosy, 1979a, b; Ciampolini et al., 1983; Thorsch and Esau, 1983).

Nuclear crystalloids are thought to be composed of proteins rich in aromatic amino acids and possibly poor in basic amino acids (Weintraub et al., 1971) or of basic proteins possibly of the histone type (Menicanti, 1972). The nuclear paracrystals of the *P. setiferum* gametophytes can be inferred to consist of proteins poor in basic amino acids, because they are not stained by Ponceau 2R (Levine, 1940).

In plants, nuclear crystalloids have been postulated to consist of reserve proteins (Frey-Wyssling and Muhlethaler, 1965). In line with this, in the leaves of Pteridophyta, these nuclear components have been assumed to "have special importance during the production of the spores" (Fabbri and Menicanti, 1970). The present findings neither support nor disprove the reserve role of the nuclear crystalloids in the *P. setiferum* gametophyte. However, cells containing these inclusions have very large vacuoles with aggregates of compact electron opaque material, few free ribosomes, no rough endoplasmic reticu-



FIGS. 1–5. Shape and structure of nuclear paracrystals of *Polystichum setiferum*. 1, 2) Mature gametophyte cells that are not adjacent to antheridia and archegonia possess single, sharp-profiled, polyhedral, nuclear crystalloids. N=nucleus; PL=plastid; scale bar=1 μm. 3, 4) Fine, very close-meshed reticulum structure of nuclear gametophyte crystalloids. Scale bar=0.2 μm. 5) Gametophyte cells with nuclear paracrystals have very large vacuoles (V) with compact electron opaque materials (arrows). The cytoplasm is a thin peripheral layer poor in organelles. Scale bar=4 μm.

lum, and few small mitochondria (Fig. 5), thus suggesting low metabolic activity, whereas the gametophyte cells adjacent to antheridia and archegonia show signs of active metabolism and have no nuclear crystalloids (Muccifora and Gori, unpublished). Hence, the buildup of the latter may be associated both with decreased nuclear and cytoplasmic activities. Even so, the crystalloids of *P. setiferum* gametophytes may consist of stored nuclear proteins, e.g., enzymes, that are not needed for the time being and are packed where they eventually will be required.

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